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Extension - 228

Responsive to the second written opinion dated August 23, 2004:

A new set of claims is submitted which should be the basis for the further prosecution.

The feature of claim 5 has been incorporated into claims 1 and 3 and 15. Former claim 5 has been deleted and claims 6 to 15 have been renumbered to claims 5 to 14. All other claims are unamended.

According to the Office Action, the present invention does not involve an inventive step over combination of D3 with D1 or D2.

The amended claims describe the synthesis of an oligonucleotide comprising synthesis cycles using solid supported reagents.

As described on page 9 of the application, the present invention is used to elongate the synthesis products stepwise.

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D3, US 5,808,042, discloses a method for removing the 5′-DMT group from an 5′-DMT oligonucleotide. D3 relates to the removal of the 5′-DMT group in the final purification; see for example column 2, lines 41 to 42: "*The present invention provides the new method of detritylating chemically synthesized oligonucleotides*", column 4, lines 18 to 20 "*used to detritylate large batches of oligonucleotides resulting from large scale synthesis*". Column 4, lines 28 to 31 describes the cleavage from solid supports, removal of base and phosphate protecting groups and subjection of the DMT-on oligonucleotide to preparative HPLC.

There is no hint or mentioning to use a solid supported reagent for the removal of the DMT during synthesis cycles. Therefore, D3 does not teach step d) as used in the method presently claimed.

Therefore, neither D1, D2 or D3 nor a combination of them discloses or suggests a complete solution phase synthesis using solid supported reagents, thereby making it possible to avoid complicated purification steps, specially chromatographic purification; see page 1, starting from line 27 of the present application.

The present invention overcomes drawbacks in prior art coming from the problem of purification of the reaction media during the repeated synthesis cycles in solution phase synthesis. This problem is overcome by the present invention.

It is kindly requested to confirm novelty, inventive step and industrial applicability for all claims.

The Patent Attorney

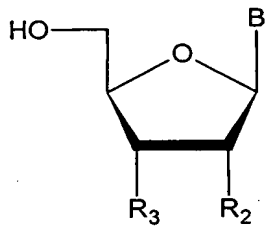
(Dr. Schreiber)

Enclosure: /

Claims

1. A method for preparing an oligonucleotide comprising the steps of

a) providing a 3'-protected compound having the formula:



wherein

B is a heterocyclic base

R_2 is H, a protected 2'-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4´-O2´methylen linkage

R_3 is OR'_3, $NHR"_3$, $NR"_3R"'_3$, a 3'-protected nucleotide or a 3'-protected oligonucleotide,

R'_3 is a hydroxyl protecting group,

$R"_3$, $R"'_3$ are independently an amine protecting group,

b) reacting said compound with a nucleotide derivative having a 5'-proctection group in the presence of a solid supported activator to give an elongated oligonucleotide with a P(III)-internucleotide bond

c) optionally processing the elongated oligonucleotide with a P(III)-internucleotide bond by either or both of steps c1) and c2) in any sequence

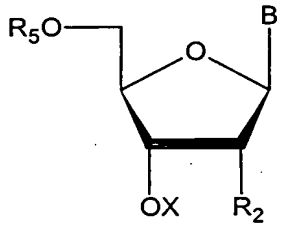
c1) capping preferably by reacting with a solid supported capping agent

c2) oxidizing preferably by reacting the oligonucleotide with a solid supported oxidizing reagent

d) removing the 5'-protection group by treatment with a solid supported agent or removing the 5´-protection group with a removal agent followed by addition of a solid supported scavenger.

e) repeating steps a) to d) at least once.

2. The method of claim 1, wherein the nucleotide derivative having a 5′-proctection group of step b) has the following formula:



wherein

X is a P(III)-function

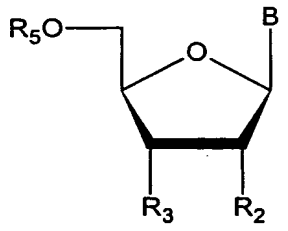
B is a heterocyclic base

R_2 is H, a protected 2′-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4′-O2′methylen linkage

R_5 is a hydroxyl protecting group, a 5′-protected nucleotide or a 5′-protected oligonucleotide.

3. A method for preparing an oligonucleotide comprising the steps of

a) providing a 5′-protected compound having the formula:



wherein

B is a heterocyclic base

R_2 is H, a protected 2′-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4′-O2′methylen linkage

R_3 is OH, NH_2

R_5 is a hydroxyl protecting group, a 5'-protected nucleotide or a 5'-protected oligonucleotide

b) reacting said compound with a nucleotide derivative having a 3'-proctection group in the presence of a solid supported activator to give an elongated oligonucleotide with a P(III)-internucleotide bond

c) optionally processing the elongated oligonucleotide with a P(III)-internucleotide bond by either or both of steps c1) and c2) in any sequence

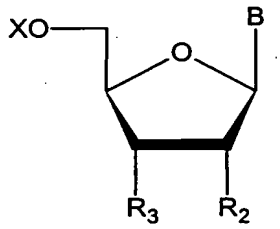
c1) capping, preferably by reacting with a solid supported capping agent

c2) oxidizing, preferably by reacting the oligonucleotide with a solid supported oxidizing reagent

d) removing the 3'-protection group by treatment with a solid supported agent or removing the 3´-protection group with a removal agent followed by addition of a solid supported scavenger.

e) repeating steps a) to d) at least once.

4. The method of claim 3, wherein the nucleotide derivative having a 3'-proctection group has the following formula:



wherein

X is a P(III)-function

B is a heterocyclic base

R_2 is H, a protected 2'-hydroxyl group, F, a protected amino group, an O-alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4´-O2´methylen linkage

R_3 = OR'_3, NHR''_3, $NR''_3R'''_3$, a 3'-protected nucleotide or a 3'-protected oligonucleotide,

R'_3 is a hydroxyl protecting group,

R''_3, R'''_3 are independently an amine protecting group,

R'_3 is a hydroxyl protecting group, a 3'-protected nucleotide or a 3'-protected oligonucleotide.

5. The method of any one of claims 1 to 4, wherein the nucleotide derivative of step b) is a phosphoramidite or a H-phosphonate.

6. The method of any one of steps 1 to 5, wherein the solid supported activator of step b) is selected from the group consisting of a solid support bearing a pyridinium salt, a cation exchange solid support with an optionally substituted pyridinium, a cation exchange solid support with an optionally substituted imidazolium salt, a solid support bearing an optionally substituted azole (imidazol, triazole, tetrazole), a salt of a weak base anion exchange resin with a strong acid, a weak cation exchange resin (carboxylic) in its protonated form, a solid support bearing an optionally substituted phenol, a solid support bearing a carboxylic acid chloride/bromide, a sulfonic acid chloride/bromide, a chloroformate, a bromoformate, a chlorosulfite, a bromosulfite, a phosphorochloridate, a phosphorbromidate and a solid support bound carbodiimide.

7. The method of any one of claims 1 to 6, wherein the solid supported oxidizing reagent is selected from the group consisting of solid supported periodates, permanganates, osmium tetroxides, dichromates, hydroperoxides, substituted alkylamine oxides, percarboxylic acid and persulfonic acid.

8. The method of any one of claims 1 to 7, wherein the oxidizing is a sulfurization.

9. The method of claim 8, wherein the solid supported oxidizing reagent is selected from the group consisting of a solid supported tetrathionate, a solid supported alkyl or aryl sulfonyl disulfide, a solid supported optionally substituted dibenzoyl tetrasulfide, a solid supported bis(akyloxythio-

carbonyl)tetrasulfide, a solid supported optionally substituted phenylacetyl disulfide, a solid supported N-[(alkyl or aryl)sulfanyl] alkyl or aryl substituted succinimide and a solid supported (2-pyridinyldithio) alkyl or aryl.

10. The method of any one of claims 1 to 9, wherein the solid supported capping agent is a solid supported activated acid, preferably a carboxylic acid chloride, carboxylic acid bromide, azolide, substituted azolide, anhydride or chloroformate or phosphorochloridate, or a solid supported phosphoramidite, or a solid supported H-phosphonate monoester.

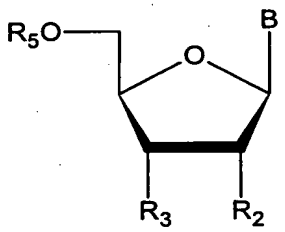
11. The method of any one of claims 1 to 10, wherein the 5′-protection is a dimethoxytrityl group (DMTr) or a monomethoxytrityl group (MMTr) and the solid supported agent of step d) is an cationic ion exchanger resin in the H^+ form or solid supported ceric ammonium nitrate.

12. The method of any one of claims 1 to 11, wherein the 3′-protection is a silyl group and the solid supported agent of step d) is an anionic ion exchanger resin in the F-form or the 3′-proctection is levulinic acid and the solid supported agent of step d) is a solid supported hydrazine or a solid supported hydrazinium.

13. Use of a solid supported sulfurization agent consisting of solid supported amine and a tetrathionate having the formula S_4O_6 or a cyanoethylthiosulfate ($NC\text{-}CH_2\text{-}CH_2\text{-}S\text{-}SO_3^-$) for sulfurization of oligonucleotides.

14. A method for preparing an oligonucleotide comprising the steps of

a) providing a compound having the formula:



wherein

B is a heterocyclic base

R_2 is H, a protected 2'-hydroxyl group, F, a protected amino group, an O-

alkyl group, an O-substituted alkyl, a substituted alkylamino or a C4´-O2´methylen linkage

and

R_3 is OR'_3, NHR''_3, $NR''_3R'''_3$,

a protected nucleotide or a protected oligonucleotide and R_5 is a P(III) function

R'_3 is a hydroxyl protecting group,

R''_3, R'''_3 are independently an amine protecting group,

or

R_5 is a hydroxyl protecting group, a protected nucleotide or a protected oligonucleotide and R_3 is a P(III) function

b) reacting said compound with a nucleotide derivative having a 3' or 5'-free OH-group in the presence of a solid supported activator to give an elongated oligonucleotide with a P(III)-internucleotide bond

c) optionally processing the elongated oligonucleotide with a P(III)-internucleotide bond by either or both of steps c1) and c2) in any sequence

c1) capping by reacting with a solid supported capping agent

c2) oxidizing by reacting the oligonucleotide with a solid supported oxidizing reagent

d) removing the 3' or 5'-protection group by treatment with a solid supported agent or removing the 5´-protection group with a removal agent followed by addition of a solid supported scavenger.

e) repeating steps a) to d) at least once.